

DESCRIPTION

Source *Spodoptera frugiperda*, Sf9 (baculovirus)-derived
Accession # NM_003161

N-terminal Sequence Analysis Using an N-terminal His tag

SPECIFICATIONS

SDS-PAGE 76 kDa

Activity The activity of p70S6K is typically 65-89 nmol/min/mg using a synthetic peptide substrate (CKRRRLASLR) (see Activity Assay Protocol).

Purity >70%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.

Formulation Supplied in 50 mM sodium phosphate (pH 7.0), 300 mM NaCl, 150 mM imidazole, 0.1 mM PMSF, 0.25 mM DTT, and 25% glycerol.
See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Active Kinase - Active p70S6K (0.1 µg/µL) diluted with Kinase Dilution Buffer. Note: These are suggested working dilutions. *Optimal dilutions should be determined by each laboratory for each application.*
- Kinase Assay Buffer I, pH 7.2 - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM MgCl₂, 5 mM EGTA, 2 mM EDTA.
Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- Kinase Dilution Buffer, pH 7.2 - Kinase Assay Buffer I diluted 5-fold with distilled or deionized water.
- 10 mM ATP Stock Solution - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I.
- [³³P]-ATP Assay Cocktail - Prepare 250 mM [³³P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 µL of 10 mM ATP Stock Solution, 100 µL of [³³P]-ATP (1 mCi/100 mL), and 5.75 mL of Kinase Assay Buffer I
- Substrate - S6K synthetic peptide substrate (CKRRRLASLR) diluted in distilled or deionized water to a final concentration of 1.0 mg/mL.

Assay

1. Thaw the [³³P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active p70S6K, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.
 - a. Diluted Active p70S6K: 10 µL
 - b. Substrate (1 mg/mL Stock Solution): 5 µL
4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled or deionized water.
5. Initiate the reaction by the addition of 5 µL [³³P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation period, terminate the reaction by spotting 20 µL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (add 10 mL of phosphoric acid to 990 mL of distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5 µL [³³P]-ATP/pmol of ATP (in 5 µL of a 250 µM ATP stock solution, i.e. 1250 pmol)

Calculation of Kinase Specific Activity (SA) (pmol/minutes/µg or nmol/minutes/mg)

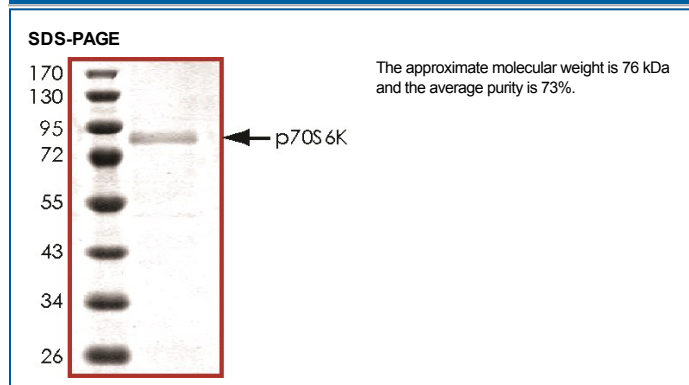
Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]

PREPARATION AND STORAGE

Shipping The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage This product is stable at ≤ -70° C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

DATA



BACKGROUND

p70S6K is responsible for the phosphorylation of 40S ribosomal protein S6 and is ubiquitously expressed in human adult tissues. p70S6K is activated by serum stimulation and this activation is inhibited by wortmannin and rapamycin. p70S6K activity undergoes changes in the cell cycle and increases 20-fold in G₁ cells released from G₀ (2). p70S6K activation requires sequential phosphorylations at proline-directed residues in the putative autoinhibitory pseudosubstrate domain, as well as at T389, a site phosphorylated by phosphoinositide-dependent kinase 1 (PDK-1) (1, 2).

References:

1. Ferrari, S. *et al.* (1994) Crit. Rev. Biochem. Mol. Biol. **29**:385.
2. Edelman, H.M. *et al.* (1996) J. Biol. Chem. **271**:963.